```
E4            1      AROC (PASTEURELLA MULTOCIDA STRAIN IL1403 CLONE PM70 GENE
AR
                     OC)/CN
E5            1      AROCAN/CN

=> s e4;e ompf/cn 5
L1            1 "AROC (PASTEURELLA MULTOCIDA STRAIN IL1403 CLONE PM70 GENE
AROC)
                "/CN


E1            1      OMPDECASE/CN
E2            1      OMPEP/CN
E3            0 --> OMPF/CN
E4            1      OMPF-LIKE PORIN (BUCHNERA STRAIN APS GENE OMPF)/CN
E5            1      OMPH (PASTEURELLA MULTOCIDA STRAIN IL1403 CLONE PM70 GENE
OM
                     PH-1)/CN

=> s e4;e ompc/cn 5
L2            1 "OMPF-LIKE PORIN (BUCHNERA STRAIN APS GENE OMPF)"/CN


E1            1      OMPANYT/CN
E2            1      OMPB2 (OUTER MEMBRANE PROTEIN B2) (BRANHAMELLA CATARRHALIS
S
                     TRAIN 56 GENE COPB)/CN
E3            0 --> OMPC/CN
E4            1      OMPDECASE/CN
E5            1      OMPEP/CN

=> s (aroa or arod or aroe or pur or htra or gale or cya or crp or phop or
sura)/cn
              0 AROA/CN
              0 AROD/CN
              0 AROE/CN
              1 PUR/CN
              0 HTRA/CN
              0 GALE/CN
              2 CYA/CN
              4 CRP/CN
              0 PHOP/CN
              0 SURA/CN
L3            7 (AROA OR AROD OR AROE OR PUR OR HTRA OR GALE OR CYA OR CRP OR
                PHOP OR SURA)/CN

=> fil medl,caplus,biosis,embase,wpids,jicst,scisearch
                                                  SINCE FILE      TOTAL
COST IN U.S. DOLLARS                                 ENTRY    SESSION
                                                     46.53      46.99
FULL ESTIMATED COST

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FILE 'CAPLUS' ENTERED AT 15:07:56 ON 16 MAY 2001
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=> s (aroa or arod or aroe or pur or htra or gale or cya or crp or phop or sura or l1 or l2 or l3 or aroc or ompf or ompc)
L4 12548 FILE MEDLINE
L5 8867 FILE CAPLUS
L6 9047 FILE BIOSIS
L7 12037 FILE EMBASE

EXCEEDS MAXIMUM FIELD LENGTH, WILL BE SEARCHED AS 'AROC (PASTEURELLA MULTOCIDA STRAIN IL1403 CLONE PM70 /CN'
L8 1001 FILE WPIDS
L9 3447 FILE JICST-EPLUS
'CN' IS NOT A VALID FIELD CODE
L10 8367 FILE SCISEARCH

TOTAL FOR ALL FILES
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=> s l11 and non revert? mutat?
L12 1 FILE MEDLINE
L13 3 FILE CAPLUS
L14 0 FILE BIOSIS
L15 0 FILE EMBASE
L16 5 FILE WPIDS
L17 0 FILE JICST-EPLUS
L18 0 FILE SCISEARCH

TOTAL FOR ALL FILES
L19 9 L11 AND NON REVERT? MUTAT?

=> s l11 and (escherichia or salmonella or vibrio or haemophilus or neisseria or yersinia or bordetella or brucella)
L20 2681 FILE MEDLINE
L21 2945 FILE CAPLUS
L22 2760 FILE BIOSIS
L23 2171 FILE EMBASE
L24 76 FILE WPIDS

Prepared by M. Hale 308-4258

L25 127 FILE JICST-EPLUS
L26 2178 FILE SCISEARCH

TOTAL FOR ALL FILES
L27 12938 L11 AND (ESCHERICHIA OR SALMONELLA OR VIBRIO OR HAEMOPHILUS OR NEISSERIA OR YERSINIA OR BORDETELLA OR BRUCELLA)

=> s l27 and vaccin?
L28 377 FILE MEDLINE
L29 376 FILE CAPLUS
L30 349 FILE BIOSIS
L31 329 FILE EMBASE
L32 45 FILE WPIDS
L33 2 FILE JICST-EPLUS
L34 367 FILE SCISEARCH

TOTAL FOR ALL FILES
L35 1845 L27 AND VACCIN?

=> s l35 and (non revert? or nonrevert?)(2a)mutat?
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L37 4 FILE CAPLUS
L38 0 FILE BIOSIS
L39 1 FILE EMBASE
L40 5 FILE WPIDS
L41 0 FILE JICST-EPLUS
L42 0 FILE SCISEARCH

TOTAL FOR ALL FILES
L43 11 L35 AND (NON REVERT? OR NONREVERT?)(2A) MUTAT?

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PROCESSING COMPLETED FOR L43
L44 8 DUP REM L43 (3 DUPLICATES REMOVED)

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L44 ANSWER 1 OF 8 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 1
1999:626318 Document No. 131:253344 Bacteria attenuated by a **non-reverting mutation** in each of the **aroC**, **ompF** and **ompC** genes, useful as **vaccines**.
Chatfield, Steven Neville (Peptide Therapeutics Limited, UK). PCT Int. Appl. WO 9949026 A1 19990930, 69 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-GB935 19990325. PRIORITY: GB 1998-6449 19980325.

AB The invention provides a bacterium attenuated by a **non-reverting mutation** in each of the **aroC** gene, the **ompF** gene and the **ompC** gene. The bacterium is useful as a **vaccine**. The bacterium may, for example, be an

attenuated strain of E. coli useful in **vaccination** against diarrhea. Thus, the design of deletions and construction of plasmids is described for removal of the entire open reading frame of target **aroC**, **ompC**, and **ompF** genes from the E1392/75/2A strain of enterotoxigenic E. coli. The attenuated **vaccine** strain (.DELTA.**aroc**/.DELTA.**ompc** /.DELTA.**ompF**) is well tolerated in healthy adult volunteers and colonizes the intestine in a manner consistent with its utility as an oral **vaccine** to protect against travelers diarrhea. It has also been demonstrated to elicit a specific mucosal immune response.

L44 ANSWER 2 OF 8 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 2
1999:388086 Document No. 131:43576 **Vaccines** containing attenuated bacteria. Chatfield, Steven Neville; Sydenham, Mark; Dougan, Gordon (Medeva Europe Limited, UK). PCT Int. Appl. WO 9929342 A1 19990617, 53 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1998-GB3680 19981210. PRIORITY: GB 1997-26233 19971211.

AB The invention relates to a **vaccine** comprising a bacterium attenuated by a **non-reverting mutation** in a gene, e.g. **surA** gene and gene for parvulin (peptidyl-prolyl cis-trans isomerase), encoding a protein which promotes folding of extracytoplasmic proteins. Such mutations were initially identified as being useful in **vaccines** from a bank of randomly inserted, transposon mutants in which attenuation was detd. as a redn. in virulence of the organism in the mouse model of infection. Site directed mutation of the gene results in a strain which shows at least 4 logs of attenuation when delivered both orally and i.v. Animals **vaccinated** with such a strain are protected against subsequent challenge with the parent wild type strain. Finally, heterologous antigens such as the non-toxic and protective, binding domain from tetanus toxin, fragment C, can be delivered via the mucosal immune system using such strains of bacteria. This results in the induction of a fully protective immune response to subsequent challenge with native tetanus toxin.

L44 ANSWER 3 OF 8 CAPLUS COPYRIGHT 2001 ACS
1999:439288 Document No. 131:69279 Using plasmid-borne complementing alleles of chromosomal genes to ensure stability of cloning vectors during propagation in bacterial hosts. Morsey, Mohamad A. (Biostar Inc., Can.). U.S. US 5922583 A 19990713, 27 pp., Cont.-in-part of U.S. Ser. No. 564,973. (English). CODEN: USXXAM. APPLICATION: US 1996-732612 19961016. PRIORITY: US 1995-548059 19951017; US 1995-564973 19951130.

AB A method of stabilizing plasmid vectors for stable, high copy no. replication of the vector in a microbial host using a plasmid-borne gene complementing a mutation in the host chromosomal genome to minimize plasmid loss without the use of antibiotics. The mutation in the host

chromosome is a **non-revertible mutation**, such as a deletion, leading to either accumulation of a toxin, such as a toxic metabolite; auxotrophy, or loss of a required intracellular protein that does not lead to a secreted product. If the DNA on the plasmid is to be used in therapeutic applications or for administration to eukaryotes, the genetic material will have no functional or structural equiv. in eukaryotic cells, and will not result in prodn. of mRNA or a polypeptide that acts on any eukaryotic cell component. Any peptide produced is, desirably, not toxic to the bacterial cells. **Escherichia** coli hosts with deletions in the **galE** and galT genes involved in the synthesis of the peptidoglycan colanic acid or in the murF gene involved in peptidoglycan synthesis were constructed. Plasmids carrying the murF gene under control of the murE promoter were constructed. Plasmids carrying the murF gene in the sense orientation showed >3-fold yields of plasmid DNA in fermentors. Mice **vaccinated** with vectors carrying the murF gene showed antibody titers comparable to control plasmids carrying the same antigen gene. The murF gene did not hybridize to human DNA.

L44 ANSWER 4 OF 8 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
AN 1991-325215 [44] WPIDS
AB WO 9115572 A UPAB: 20000105
Microorganisms (I) for use in immunoprophylaxis are attenuated as a result of the presence of a mutation in the DNA sequence of the microorganism which encodes, or which regulates the expression of DNA encoding, a protein that is produced in response to environmental stress. The microorganism is opt. capable of expression DNA encoding a heterologous antigen. Also new is a **vaccine** contg. (I).

The protein is a nutrient deprivation protein, toxic stress protein, metabolic distress protein or especially a heat shock protein encoded by the **htrA** gene. The microorganism is a bacterium such as **Bordetella, Vibrio**, Haemopilus, Esherichia or especially **Salmonella**.

USE/ADVANTAGE - (I) are useful in live **vaccines** and immunoprophylaxis of e.g. salmonellosis, whooping cough, meningitis and gonorrhoea. Dosage of S. typti is 10 power 9 - 10 power 11 organisms/dose.

An attentuated form of S. typhimurium (strain 046) had log 10 ID50 of more than 9 cells, cf. the parental virulent strain C5 which had a log 10 LD50 of 6.38 cells, 28 days following oral administration.

Dwg.0/3

ABEQ ZA 9102397 A UPAB: 19930928
Microorganisms (I) for use in immunoprophylaxis are attenuated as a result of the presence of a mutation in the DNA sequence of the microorganism which encodes, or which regulates the expression of DNA encoding, a protein that is produced in response to environmental stress. The microorganism is opt. capable of expression DNA encoding a heterologous antigen. Also new is a **vaccine** contg. (I).

The protein is a nutrient deprivation protein, toxic stress protein, metabolic distress protein or esp. a heat shock protein encoded by the **htrA** gene. The microorganism is a bacterium e.g.

Bordetella, **Vibrio**, Haemopilus, Esherichia or especially **Salmonella**.

USE/ADVANTAGE - (I) are useful in live **vaccines** and immunoprophylaxis of e.g. salmonellosis, whooping cough, meningitis and gonorrhoea. Dosage of S. typti is 10 power(9)- 10 power (11) organisms/dose.

An attenuated form of S. typhimurium (strain 046) and log 10 ID50 of more than 9 cells, cf. the parental virulent strain C5 which had a log 10LD50 of 6.38 cells, 28 days following oral administration

ABEQ EP 524205 B UPAB: 19970926

A **vaccine** comprising a pharmaceutically acceptable carrier and an effective amount of a bacterium attenuated by a **non-reverting mutation** in the **htrA** gene.

Dwg.0/3

L44 ANSWER 5 OF 8 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD

AN 1990-363446 [49] WPIDS

AB EP 400958 A UPAB: 19970417

An attenuated bacteria, with a mutation in a gene concerned with regulating one or more additional genes, is new. The genes regulated encode an outer membrane protein and are porin genes. The regulating gene is Omp. R. The bacteria is gram negative and selected from **Salmonella**, **Bordetella**, Viloris, **Haemophilus** and **Escherichia** genera, pref. it is from S. typhi an A-, omp-, S. typhimium omp-, or **aroA**-, omp R- or S. dublin omp R- or **aroA**, ompR-. Opt. a second gene is also mutated, which encodes an enzyme involved in an essential auxotrophic pathway. This gene is pref. anoA, **aroC**, or **aroD**.

USE/ADVANTAGE - Bacteria attenuated in such a way that can be used as live **vaccines** in human and animal medicine. It can be used in a prophylactic treatment of a bacterial infection, in an effective dose which depends on various clinical factors. For S.typhi a dosage of 109-1011 organisms/dose is used for a 70 kg human patient. @(9pp Dwg.No.0/1)@

ABEQ EP 400958 B UPAB: 19951019

A **vaccine** formulation comprising a bacterium attenuated by a non-revering mutation in the ompR gene in admixture with a pharmaceutically acceptable excipient.

Dwg.0/0

ABEQ US 5527529 A UPAB: 19960731

A pharmaceutical composition for oral administration to a subject for inducing immunity to a pathogenic **Salmonella** bacterium, which composition comprises a pharmaceutically acceptable excipient and an attenuation form of said **Salmonella** bacterium, the attenuation being attributable to a **non-reverting mutation** in the ompR gene of said **Salmonella** bacterium.

Dwg.0/1

L44 ANSWER 6 OF 8 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD

AN 1989-309381 [42] WPIDS

CR 1985-289426 [46]; 1986-155753 [24]; 1989-206100 [28]

AB WO 8909063 A UPAB: 19960227

The following are claimed as new (A) a live Shigella strain having a requirement for at least one essential metabolite which is not available

in a mammalian host, the requirement being as a result of a **non-reverting mutation**; (B) a live Shigella strain having the following properties: non-reverting **aroD**-; Sereny negative; Congo red positive; serotype Y; sensitivity to antibiotics, and ability to grow on chemically defined media; (C) a **vaccine** strian of Shigella flexneri least one essential metabolite which is not available in a mammalian host, the requirement being as a result of a non-reverting deletion or deletion-inversion, grow on chemically defined media, are sensitive to antibiotics, are Sereny-negative and comprise the invasiveness plasmid; (D) Shigella flexneri strain SFL 114, ATCC 53755.

USE/ADVANTAGE - The auxotrophic **vaccine** strains provided have non-reverting blocks in a biosynthetic pathway which ensure that though the strains live in a host organism they are unable to be proliferated. The mutated organisms retain the same antigenic characteristics as the unmutated, virulent organisms, thus inducing a protective immune response.

Dwg.0/0

Dwg.0/0

ABEQ US 5077044 A UPAB: 19930923

Live Shigella strains, e.g. Shigella flexneri, serotypes 1a, 1b, 2a, 2b, 3a, 4a, 4b and 5 (obtd. by lysogenisation of a serotype Y with one or more bacteriophages) are new strains which require one or more essential metabolites not normally present in a mammalian host. These strains do not revert to aromatic D-(-)-aminoacids, are Sereney negative but Congo red positive, and are sensitive to numerous antibiotics.

USE - The prods. are components for **vaccines** against dysentery, but other microorganism strains can be modified in a similar manner to provide a wide range of **vaccines**. @

ABEQ US 5210035 A UPAB: 19931113

Prepn. of a live non-virulent **vaccine** from a virulent pathogenic microorganism, comprises subjecting a strain of microorganism to mutation, giving a mutated microorganism having at least two **non-reverting mutations**. **Mutations** involve at least 5 nucleotides each and result in a block in at least one biosynthetic pathway which renders the organism auxotrophic with a metabolite normally unavailable in a host. Mutations comprise at least one of a deletion, insertion or inversion. **Non-reverting mutated** microorganism is then selected for.

Also claimed is a **vaccine** comprising the mutant.

USE/ADVANTAGE - As a **vaccine** against **Salmonella** and Shigella. Does not revert to virulence.

Dwg.0/0

ABEQ EP 368966 B UPAB: 19960610

Shigella flexneri strain SFL114, ATCC Accession No. 53755, or mutants or derivatives thereof.

Dwg.0/0

ABEQ US 5643771 A UPAB: 19970806

Preparation of a live non-virulent **vaccine** from a virulent pathogenic bacterial microorganism, the **vaccine** being

substantially incapable of reverting to virulence in a vertebrate host susceptible to the microorganism, h comprises:

(a) subjecting a virulent strain of said microorganism to mutating conditions resulting in a mutated microorganism having at least two **non-reverting mutations** involving at least five nucleotides each and resulting in a block in at least one biosynthetic pathway which renders the organism auxotrophic with a requirement for a metabolite normally unavailable in a host susceptible to said microorganism, the mutations comprising at least one of deletion, insertion or inversion;

(b) selecting for **non-reverting mutated** microorganism;

(c) isolating **non-reverting mutated** microorganism to provide a living **vaccine;**

(d) introducing an expression cassette containing a DNA sequence encoding an antigen foreign to said pathogenic microorganism, under regulatory control of regulatory regions recognized by said pathogenic microorganism, into said pathogenic microorganism or mutant microorganism to produce a transformed host cell;

(e) growing said transformed host cell; and

(f) identifying and isolating transformed host cells expressing said antigen;

wherein (d), (e) and (f) may be carried out before or after any one of (a) through (c), resulting in a culture of auxotrophic, non-reverting, non-virulent mutant microorganism capable of expressing an antigen foreign to said microorganism.

Dwg.0/0

L44 ANSWER 7 OF 8 MEDLINE DUPLICATE 3

87203129 Document Number: 87203129. PubMed ID: 3106921. Live oral **Salmonella vaccines**: potential use of attenuated strains as carriers of heterologous antigens to the immune system. Dougan G; Hormaeche C E; Maskell D J. PARASITE IMMUNOLOGY, (1987 Mar) 9 (2) 151-60. Ref: 41. Journal code: OQU; 7910948. ISSN: 0141-9838. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Live attenuated strains of **salmonellae** are showing promise as live oral **vaccines** against human typhoid fever and other **Salmonella** infections of man and animals. Attenuation can be achieved by introducing genetically defined, **non-reverting mutations** into specific genes on the **Salmonella** chromosome. Mutations in the gal E or **aroA** genes of **Salmonella** inhibit the ability of the bacteria to grow in vivo, and strains carrying such lesions are effective **vaccines** against salmonellosis. Genetic determinants encoding for the expression of potentially protective antigens from heterologous, non-**Salmonella** pathogens can be readily introduced into these attenuated **Salmonella** strains. Expression of the heterologous antigen does not affect the ability of the **Salmonella** host to be used as a **Salmonella vaccine**. Mice infected orally with a **Salmonella** typhimurium **aroA vaccine** expressing the **Escherichia** coli heat-labile toxin B subunit developed both a secretory and serum antibody response to this antigen. These serum

antibodies were able to neutralise the activity of E. coli heat-labile toxin in tissue culture assays. A humoral and cell-mediated (DTH) immune response was detected against beta galactosidase, an intracellular antigen, in mice infected with an **aroA vaccine** expressing this cloned antigen. The prospects for the development of live **Salmonella vaccines** as a method for delivering heterologous antigens derived from bacteria, viruses and parasites is discussed.

L44 ANSWER 8 OF 8 CAPLUS COPYRIGHT 2001 ACS
1987:154294 Document No. 106:154294 Genetics of **Salmonella** and Shigella strains used as live **vaccines**. Stocker, B. A. D. (Sch. Med., Stanford Univ., Stanford, CA, 94305, USA). Dev. Vaccines Drugs Diarrhea, Nobel Conf. ["Recent Adv. Vaccines Drugs Diarrhoeal Dis."], 11th, Meeting Date 1985, 127-9. Editor(s): Holmgren, Jan; Lindberg, Alf; Moellby, Roland. Studentlitteratur: Lund, Swed. (English) 1986. CODEN: 55MDAX.
AB A review with 5 refs. Live **vaccines** prepd. from **Salmonella** strains having **non-reverting** transposon-generated **mutations** of gene **aroA** or deletion at purA were non-virulent and gave good protection in mice and calves.

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=> s chatfield s?/au,in and l35
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L45          33 FILE MEDLINE
L46          33 FILE CAPLUS
L47          27 FILE BIOSIS
'IN' IS NOT A VALID FIELD CODE
L48          23 FILE EMBASE
L49           5 FILE WPIDS
L50           0 FILE JICST-EPLUS
'IN' IS NOT A VALID FIELD CODE
L51          26 FILE SCISEARCH

TOTAL FOR ALL FILES
L52         147 CHATFIELD S?/AU,IN AND L35

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L58           0 FILE JICST-EPLUS
L59          26 FILE SCISEARCH

TOTAL FOR ALL FILES
L60         141 L52 NOT L43

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L61           42 DUP REM L60 (99 DUPLICATES REMOVED)
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L61 ANSWER 1 OF 42 MEDLINE DUPLICATE 1
2001021219 Document Number: 20448972. PubMed ID: 10992518. Comparison of abilities of **Salmonella** enterica serovar typhimurium **aroA aroD** and **aroA htrA** mutants to act as live vectors. Roberts M; **Chatfield S**; Pickard D; Li J; Bacon A. (Department of Veterinary Pathology, Glasgow University Veterinary School, Glasgow G61 1QH, United Kingdom.. M.Roberts@vet.gla.ac.uk) . INFECTION AND IMMUNITY, (2000 Oct) 68 (10) 6041-3. Journal code: GO7. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB We compared the ability of **Salmonella** enterica serovar Typhimurium SL1344 **aroA aroD** (BRD509) and **aroA htrA** (BRD807) mutants to act as live vectors for delivery of fragment C of tetanus toxin (FrgC). FrgC was expressed in these strains from either pTETnir15 or pTEThtrA1. BRD509FrgC(+) strains elicited approximately 2-log-higher serum anti-FrgC antibody titers than BRD807FrgC(+) strains. All mice immunized with BRD807pTEThtrA1, BRD509pTEThtrA1, and BRD509pTETnir15 (but not BRD807pTETnir15) were protected against tetanus.

L61 ANSWER 2 OF 42 MEDLINE DUPLICATE 2
2000143725 Document Number: 20143725. PubMed ID: 10678926. Phase 2 clinical trial of attenuated **Salmonella** enterica serovar typhi oral live vector **vaccine** CVD 908-**htrA** in U.S. volunteers. Tacket C O; Sztein M B; Wasserman S S; Losonsky G; Kotloff K L; Wyant T L; Nataro J P; Edelman R; Perry J; Bedford P; Brown D; **Chatfield S**; Dougan G; Levine M M. (Center for Vaccine Development, Department of Medicine, University of Maryland School of Medicine, Baltimore, Maryland, USA.. ctacket@medicine.umaryland.edu) . INFECTION AND IMMUNITY, (2000 Mar) 68 (3) 1196-201. Journal code: GO7; 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB **Salmonella** enterica serovar Typhi strain CVD 908-**htrA** is a live attenuated strain which may be useful as an improved oral typhoid **vaccine** and as a vector for cloned genes of other pathogens. We conducted a phase 2 trial in which 80 healthy adults received one of two dosage levels of CVD 908-**htrA** in a double-blind, placebo-controlled, crossover study. There were no differences in the rates of side effects among volunteers who received high-dose **vaccine** (4.5 x 10(8) CFU), lower-dose **vaccine** (5 x 10(7) CFU), or placebo in the 21 days after **vaccination,** although recipients of high-dose **vaccine** (8%) had more frequent diarrhea than placebo recipients (0%) in the first 7 days. Seventy-seven percent and 46% of recipients of high- and lower-dose **vaccines,** respectively, briefly excreted **vaccine** organisms in their stools. All blood cultures were negative. Antibody-secreting cells producing antilipopolysaccharide (LPS) immunoglobulin A (IgA) were detected in 100 and 92% of recipients of high- and lower-dose **vaccines,** respectively. Almost half the volunteers developed serum anti-LPS IgG. Lymphocyte proliferation and gamma interferon production against serovar Typhi antigens occurred in a significant proportion of **vaccinees**. This phase 2 study supports the further development of CVD 908-**htrA** as a single-dose **vaccine** against typhoid fever and as a possible live vector for oral delivery of other

vaccine antigens.

L61 ANSWER 3 OF 42 MEDLINE DUPLICATE 3
2000143713 Document Number: 20143713. PubMed ID: 10678914.
Salmonella enterica serovar typhimurium **surA** mutants are attenuated and effective live oral **vaccines**. Sydenham M; Douce G; Bowe F; Ahmed S; **Chatfield S**; Dougan G. (Medeva Vaccine Development Group, Department of Biochemistry, Imperial College of Science, Technology and Medicine, London SW7 2AZ, United Kingdom.) INFECTION AND IMMUNITY, (2000 Mar) 68 (3) 1109-15. Journal code: GO7; 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.
AB A previously described attenuated TnphoA mutant (BRD441) of **Salmonella** enterica serovar Typhimurium C5 (I. Miller, D. Maskell, C. Hormaeche, K. Johnson, D. Pickard, and G. Dougan, Infect. Immun. 57:2758-2763, 1989) was characterized, and the transposon was shown to be inserted in **surA**, a gene which encodes a peptidylprolyl-cis, trans-isomerase. A defined **surA** deletion mutation was introduced into S. enterica serovar Typhimurium C5 and the mutant strain, named S. enterica serovar Typhimurium BRD1115, was extensively characterized both in vitro and in vivo. S. enterica serovar Typhimurium BRD1115 was found to be defective in the ability to adhere to and invade eukaryotic cells. Furthermore, S. enterica serovar Typhimurium BRD1115 was attenuated by at least 3 log units when administered orally or intravenously to BALB/c mice. Complementation of the mutation with a plasmid carrying the intact **surA** gene almost completely restored the virulence of BRD1115. In addition, S. enterica serovar Typhimurium BRD1115 demonstrated potential as a **vaccine** candidate, since mice immunized with BRD1115 were protected against subsequent challenge with S. enterica serovar Typhimurium C5. S. enterica serovar Typhimurium BRD1115 also showed potential as a vehicle for the effective delivery of heterologous antigens, such as the nontoxic, protective fragment C domain of tetanus toxin, to the murine immune system.

L61 ANSWER 4 OF 42 MEDLINE DUPLICATE 4
2001043454 Document Number: 20484124. PubMed ID: 11027455. Safety and immune responses to attenuated **Salmonella** enterica serovar typhi oral live vector **vaccines** expressing tetanus toxin fragment C. Tacket C O; Galen J; Sztein M B; Losonsky G; Wyant T L; Nataro J; Wasserman S S; Edelman R; **Chatfield S**; Dougan G; Levine M M. (Center for Vaccine Development, University of Maryland School of Medicine, 685 West Baltimore Street, Baltimore, Maryland 21201, USA.. ctacket@medicine.umaryland.edu) . CLINICAL IMMUNOLOGY, (2000 Nov) 97 (2) 146-53. Journal code: C90. ISSN: 1521-6616. Pub. country: United States. Language: English.
AB Attenuated **Salmonella** enterica serovar Typhi **vaccine** strain CVD 908-**htrA** was used as a vector to deliver fragment C of tetanus toxin as a single-dose oral tetanus **vaccine** candidate to elicit protective levels of serum tetanus antitoxin. Twenty-one healthy adult volunteers received doses of 1.6 x 10(7) to 8.2 x 10(9) CFU of one of two strains, CVD 908-**htrA**(pTETnir15) or CVD 908-**htrA** (pTETlpp), which contained plasmid-encoded fragment C, with sodium bicarbonate, and the safety and immune responses to serovar Typhi antigens

and tetanus toxin were assessed. No volunteer had fever or positive blood cultures after **vaccination**, although diarrhea occurred in 3 volunteers and vomiting in 2 volunteers within 3 weeks after **vaccination**. Most volunteers excreted the **vaccine** strain in the first 72 h after **vaccination**. Three of nine volunteers who received 10(8) CFU or higher doses of the CVD 908-**htrA** (pTETlpp) construct developed rises in serum antitoxin antibodies. The serum and cellular immune responses to serovar Typhi antigens were less frequent than those previously observed in volunteers who ingested the parent strain CVD 908-**htrA**. This study demonstrates that fragment C of tetanus toxin delivered orally to volunteers in an S. Typhi vector can elicit protective levels of serum antitoxin.

L61 ANSWER 5 OF 42 CAPLUS COPYRIGHT 2001 ACS
1999:223054 Document No. 130:266359 Hepatitis B virus fusion polypeptides (tetanus toxin fused to pre-S1 antigen and/or pre-S2 antigen) and their use in the prevention or treatment of HBV infections. **Chatfield, Steven Neville** (Medeva Europe Limited, UK). PCT Int. Appl. WO 9915671 A1 19990401, 30 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1998-GB2852 19980921. PRIORITY: GB 1997-20033 19970919.
AB The present invention provides polypeptides comprising tetanus toxin fragment C, or a fragment thereof, fused to the pre-S1 region of hepatitis B virus (HBV), or a fragment thereof, and/or the pre-S2 region of HBV or a fragment thereof. The present invention also provides polynucleotides encoding the fusion polypeptides of the invention. The invention further provides vectors comprising a polynucleotide encoding a polypeptide of the invention operably linked to the promoter region of gene **htrA** and a host cell transfected with these vectors. The polypeptides, polynucleotides, and vectors may be used in the prevention or treatment of HBV infections. Still further, the invention provides a **vaccine** compn. comprising a polypeptide, polynucleotide or vector of the invention together with a pharmaceutically acceptable carrier diluent. Finally, the invention produces a method for producing antibodies which recognize epitopes within the pre-S1 and/or pre-S2 regions of HBV and use of these antibodies in treatment of HBV infections.

L61 ANSWER 6 OF 42 MEDLINE DUPLICATE 5
1999346164 Document Number: 99346164. PubMed ID: 10417142. Prior immunity to homologous and heterologous **Salmonella** serotypes suppresses local and systemic anti-fragment C antibody responses and protection from

tetanus toxin in mice immunized with **Salmonella** strains expressing fragment C. Roberts M; Bacon A; Li J; **Chatfield S.** (Department of Veterinary Pathology, Glasgow University Veterinary School, Glasgow G61 1QH, United Kingdom.. M.Roberts@vet.gla.ac.uk) . INFECTION AND IMMUNITY, (1999 Aug) 67 (8) 3810-5. Journal code: GO7; 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB We have investigated the effect of preexisting immunity to homologous (**Salmonella** typhimurium) or heterologous (S. dublin) serotypes of **Salmonella** on the ability of an attenuated S. typhimurium **aroA aroD** vector (BRD509) to immunize mice against the heterologous antigen fragment C (FrgC). We studied two strains, BRD847 and BRD937, expressing FrgC carried on plasmids that differ only with respect to the promoter controlling FrgC expression, the nirB promoter in the case of BRD847 and the **htrA** promoter in the case of BRD937. Mice were preimmunized orally with S. typhimurium BRD509, S. dublin **aroA aroD** (BRD620), or saline. Forty-four days later, they were immunized orally with BRD847 or BRD937. Prior immunity to S. typhimurium severely depressed the serum immunoglobulin G (IgG) and IgA anti-FrgC response in both BRD847- and BRD937-immunized mice. Mice with existing immunity to S. dublin also had lower IgG anti-FrgC geometric mean titers (GMTs) than did mice preimmunized with saline, but this difference was significant only in the case of mice immunized with BRD937. However, in nonimmune mice or in mice preimmunized with S. typhimurium or S. dublin, the anti-FrgC IgG GMTs were always higher in mice in the BRD937 groups than in the equivalent BRD847 groups. This is reflected in the effect of prior immunity on the ability of oral immunization with BRD847 or BRD937 to protect mice from challenge with a lethal dose of tetanus toxin. All of the mice preimmunized with saline and then immunized with BRD847 or BRD937 survived challenge. Only 20% of the animals immunized with BRD847 and 60% of the mice in the BRD937 group survived tetanus toxin challenge if they were preimmunized with BRD509. Preexisting immunity to S. dublin did not affect the ability of BRD937 to immunize mice against tetanus, but it did reduce the efficiency of BRD847: only 60% percent of the mice survived challenge. The intestinal secretory IgA responses to FrgC were very similar in the BRD847 and BRD937 groups. Prior immunity did depress the IgA anti-FrgC titers but only significantly so in the mice preimmunized with BRD509. These results show that preexisting **Salmonella** immunity, particularly to homologous serotypes, can severely compromise the ability of live **Salmonella** vectors to deliver heterologous antigens to the mammalian immune system. However, the results also indicate that this may be overcome by the design of more powerful in vivo expression systems.

L61 ANSWER 7 OF 42 MEDLINE DUPLICATE 6

1999115546 Document Number: 99115546. PubMed ID: 9916080.

Characterization of candidate live oral **Salmonella** typhi **vaccine** strains harboring defined mutations in **aroA, aroC**, and **htrA**. Lowe D C; Savidge T C; Pickard D; Eckmann L; Kagnoff M F; Dougan G; **Chatfield S N.** (Department of

Cellular Physiology, The Babraham Institute, Babraham, Cambridge CB2 4AT, Imperial College of Science, Technology and Medicine, London SW7 2AY, United Kingdom.) INFECTION AND IMMUNITY, (1999 Feb) 67 (2) 700-7. Journal code: GO7; 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB The properties of two candidate **Salmonella** typhi-based live oral typhoid **vaccine** strains, BRD691 (S. typhi Ty2 harboring mutations in **aroA** and **aroC**) and BRD1116 (S. typhi Ty2 harboring mutations in **aroA**, **aroC**, and **htrA**), were compared in a number of in vitro and in vivo assays. BRD1116 exhibited an increased susceptibility to oxidative stress compared with BRD691, but both strains were equally resistant to heat shock. Both strains showed a similar ability to invade Caco-2 and HT-29 epithelial cells and U937 macrophage-like cells, but BRD1116 was less efficient at surviving in epithelial cells than BRD691. BRD1116 and BRD691 were equally susceptible to intracellular killing within U937 cells. Similar findings were demonstrated in vivo, with BRD1116 being less able to survive and translocate to secondary sites of infection when inoculated into the lumen of human intestinal xenografts in SCID mice. However, translocation of BRD1116 to spleens and livers in SCID mice occurred as efficiently as that of BRD691 when inoculated intraperitonally. The ability of BRD1116 to increase the secretion of interleukin-8 following infection of HT-29 epithelial cells was comparable to that of BRD691. Therefore, loss of the **HtrA** protease in S. typhi does not seem to alter its ability to invade epithelial cells or macrophages or to induce proinflammatory cytokines such as IL-8 but significantly reduces intracellular survival in human intestinal epithelial cells in vitro and in vivo.

L61 ANSWER 8 OF 42 CAPLUS COPYRIGHT 2001 ACS

1999:25078 Oral **vaccination** against tetanus: comparison of the immunogenicities of **Salmonella** strains expressing fragment C from the nirB and **htrA** promoters. Roberts, Mark; Li, Jingli; Bacon, Andrew; **Chatfield, Steven** (Dep. of Vet. Pathol., Glasgow Univ. Vet. Sch., Glasgow, G61 1QH, UK). Infect. Immun., 67(1), 468 (English) 1999. CODEN: INFIBR. ISSN: 0019-9567. Publisher: American Society for Microbiology.

AB Unavailable

L61 ANSWER 9 OF 42 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.

1999016730 EMBASE Erratum: Oral **vaccination** against tetanus: Comparison of the immunogenicities of **Salmonella** strains expressing fragment C from the nirB and **htrA** promoters (Infection and Immunity (1998) 66:7 (3080-3087)). Roberts M.; Li J.; Bacon A.; **Chatfield S.**. M. Roberts, Department of Veterinary Pathology, Glasgow University Veterinary School, Glasgow G61 1QH, United Kingdom. Infection and Immunity 67/1 (468) 1999. ISSN: 0019-9567. CODEN: INFIBR. Pub. Country: United States. Language: English.

L61 ANSWER 10 OF 42 SCISEARCH COPYRIGHT 2001 ISI (R)

1999:41913 The Genuine Article (R) Number: 152EV. Oral **vaccination** against tetanus: Comparison of the immunogenicities of **Salmonella** strains expressing fragment C from the nirB and **htrA** promoters (vol 66, pg 3080, 1998). Roberts M (Reprint); Li J L; Bacon A; **Chatfield S**. UNIV GLASGOW, SCH VET, DEPT VET PATHOL, GLASGOW G61 1QH, LANARK, SCOTLAND (Reprint); UNIV LONDON IMPERIAL COLL SCI TECHNOL & MED, DEPT BIOCHEM, MEDEVA, VACCINE RES UNIT, LONDON SW7 2AZ, ENGLAND. INFECTION AND IMMUNITY (JAN 1999) Vol. 67, No. 1, pp. 468-468. Publisher: AMER SOC MICROBIOLOGY. 1325 MASSACHUSETTS AVENUE, NW, WASHINGTON, DC 20005-4171. ISSN: 0019-9567. Pub. country: SCOTLAND; ENGLAND. Language: English.

L61 ANSWER 11 OF 42 MEDLINE DUPLICATE 7
1998298022 Document Number: 98298022. PubMed ID: 9632569. Oral **vaccination** against tetanus: comparison of the immunogenicities of **Salmonella** strains expressing fragment C from the nirB and **htrA** promoters. Roberts M; Li J; Bacon A; **Chatfield S.** (Department of Veterinary Pathology, Glasgow University Veterinary School, Glasgow G61 1QH, United Kingdom.. M.Roberts@vet.gla.ac.uk) . INFECTION AND IMMUNITY, (1998 Jul) 66 (7) 3080-7. Journal code: GO7; 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB We have found the in vivo-regulated nirB promoter (PnirB) to be effective for directing expression of a number of antigens in **salmonella** in vivo. We wished to determine if other in vivo-regulated promoters have utility for antigen expression in **salmonella** and to compare the effectiveness of these promoters with that of PnirB. To this end, we have devised a scheme that allows the promoter element of the PnirB-fragment C plasmid pTETnir15 to be swapped with other promoters of interest. We demonstrate the usefulness of this system by replacing PnirB with PhtrA to create plasmid pTEThtrA1. **htrA** is a stress response gene that is required for virulence of **salmonella** in mice and survival within macrophages. Expression of fragment C in **Salmonella** typhimurium BRD509 (**aroA aroD**) harboring pTEThtrA1 (strain BRD937) correlated with growth temperature in vitro. A comparison was made of the immune responses to fragment C elicited in mice immunized orally with BRD937 or BRD847 (BRD509/pTETnir15) or subcutaneously with purified fragment C plus alhydrogel. High levels of anti-fragment C antibodies that persisted for at least 12 weeks were present in all groups of mice. **Vaccination** with BRD937 was the most effective means of immunization: the serum immunoglobulin G (IgG), IgA, and IgM anti-fragment C titers were higher in the BRD937-immunized mice throughout the duration of the study than in mice in the other groups. The kinetics of the serum anti-fragment C responses were different in different groups. The response was most rapid in the BRD937 group, with the titers almost at peak levels at 2 weeks postimmunization. Only the mice immunized with BRD937 or BRD847 developed an intestinal IgA response to fragment C. Again, the response was superior in the BRD937 group. The peak of the intestinal response was delayed with respect to the serum response. Analysis of the IgG subtype

response to fragment C revealed a dominant IgG2a response in the **salmonella**-immunized mice, indicating a type 1 helper T-cell response to fragment C, whereas the major subtype in the group parenterally immunized with fragment C plus alhydrogel was IgG1. The IgG1/IgG2a ratio was much higher in sera of BRD937-immunized mice than in sera of BRD847-immunized mice. At 15 to 20 weeks after immunization, the mice immunized with BRD937 or BRD847 were solidly immune to tetanus toxin and **salmonella**. The immune responses to fragment C seen in mice immunized with BRD937 are the strongest we have observed and indicate that the **htrA** promoter may be very useful for expressing foreign antigens in **salmonella vaccine** strains.

L61 ANSWER 12 OF 42 MEDLINE DUPLICATE 8
1998230472 Document Number: 98230472. PubMed ID: 9570545. Protective effect on Leishmania major infection of migration inhibitory factor, TNF-alpha, and IFN-gamma administered orally via attenuated **Salmonella** typhimurium. Xu D; McSorley S J; Tetley L; **Chatfield S**; Dougan G; Chan W L; Satoskar A; David J R; Liew F Y. (Department of Immunology, University of Glasgow, United Kingdom.) JOURNAL OF IMMUNOLOGY, (1998 Feb 1) 160 (3) 1285-9. Journal code: IFB; 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB The genes encoding murine macrophage migration inhibitory factor (MIF), IL-2, IFN-gamma or TNF-alpha were cloned individually into an expression plasmid under the control of the inducible promoter nirB and transfected into the **aroA- aroD-** deletion mutant strain of **Salmonella** typhimurium (BRD509). These S. typhimurium derivatives (henceforward called constructs and termed GIDMIF, GIDIL2, GIDIFN and GIDTNF) expressed their respective cytokines in vitro under anaerobic conditions and stably colonized BALB/c mice up to 14 days after oral administration. The highly susceptible BALB/c mice that had received the constructs orally and that had been subsequently infected via the footpad with Leishmania major, developed significantly reduced disease compared with control mice administered the untransfected **Salmonella** strain (BRD509). Importantly, a combination of GIDMIF, GIDIFN, and GIDTNF administered orally after L. major infection was able to significantly limit lesion development and reduced parasite loads by up to three orders of magnitude. Spleen and lymph node cells of mice administered this combination expressed markedly higher levels of inducible nitric oxide synthase (iNOS) compared with those from mice receiving an equivalent dose of the control strain of **Salmonella** (BRD509). These data therefore demonstrate the feasibility of therapeutic treatment in an infectious disease model using cytokines delivered by attenuated **Salmonella**. The protective effect observed correlates with the induction of inducible nitric oxide synthase in vivo.

L61 ANSWER 13 OF 42 MEDLINE
1998269891 Document Number: 98269891. PubMed ID: 9607008. Immune responses in calves immunised orally or subcutaneously with a live **Salmonella** typhimurium aro **vaccine**. Villarreal-Ramos B; Manser J; Collins R A; Dougan G; **Chatfield S N**; Howard C J. (Institute for Animal Health, Newbury, Berkshire, UK.. Bernardo.Villarreal@BBSRC.AC.UK) . VACCINE, (1998 Jan) 16 (1) 45-54.

Journal code: X6O; 8406899. ISSN: 0264-410X. Pub. country: ENGLAND: United
Kingdom. Language: English.

AB **Salmonella** aro **vaccines** are able to confer solid protection against homologous virulent challenge in several animal species. Calves were protected against virulent S. typhimurium challenge following administration of a single oral dose of live BRD562 **vaccine**. Immune responses elicited by the S. typhimurium aro **vaccine** strain BRD562 were studied following administration to calves by either the oral or subcutaneous route. Serum antibodies to **Salmonella** polypeptides, following oral or subcutaneous **vaccination**, were detected by immunoblotting and the route of inoculation found to affect both the antibody isotype and the antigens detected. Oral, but not subcutaneous, immunisation induced bovine serum IgA antibodies against **Salmonella** antigens of 30 kDa and 65 kDa and bovine IgG2 antibodies against a 35 kDa antigen. Subcutaneous **vaccination** triggered responses against antigens of 52 kDa, 54 kDa and 57 kDa which were not detected by immune plasma of animals immunised orally. Antibody responses to LPS were poor in animals inoculated by either route. Subcutaneous **vaccination** elicited T-cell responses against **Salmonella** antigens as measured by in vitro peripheral blood cell thymidine incorporation. These studies show that the S. typhimurium **vaccine** strain BRD562 is capable of inducing both humoral and cellular immune responses. Further studies are necessary to identify the nature of the antigens responsible for protection. Oral or subcutaneous inoculation of BRD562(pTETnir15) failed to induce serum antibodies against the fragment C of tetanus toxin (TetC) but was effective in mice. Oral **vaccination** with this recombinant **vaccine** induced mucosal IgA against TetC. This is the first time that **Salmonella** recombinant **vaccines** have been shown to successfully elicit antibodies against a guest antigen in cattle after one single oral inoculation.

L61 ANSWER 14 OF 42 MEDLINE DUPLICATE 9

97162309 Document Number: 97162309. PubMed ID: 9009296. Safety of live oral **Salmonella** typhi **vaccine** strains with deletions in **htrA** and **aroC aroD** and immune response in humans. Tacket C O; Sztein M B; Losonsky G A; Wasserman S S; Nataro J P; Edelman R; Pickard D; Dougan G; **Chatfield S N**; Levine M M. (Department of Medicine, University of Maryland School of Medicine, Baltimore 21201, USA.. ctacket@umppal.ab.umd.edu) . INFECTION AND IMMUNITY, (1997 Feb) 65 (2) 452-6. Journal code: GO7; 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB A single-dose, oral **Salmonella** typhi **vaccine** strain has been sought as a carrier or vector of cloned genes encoding protective antigens of other pathogens. Such a hybrid **vaccine**, administered orally, would stimulate immune responses both at the mucosal surface and in the systemic compartment and would potentially provide protection against multiple pathogens. S. typhi CVD 908 and CVD 906, which harbor deletions in **aroC** and **aroD**, were further engineered by deletion in **htrA** to produce strains CVD 908-**htrA** and CVD 906-**htrA**, which are unable to sustain growth and are severely impaired in their ability to survive in host tissues. These strains were fed to humans at doses of 5 x 10(7) to 5 x 10(9) CFU with

buffer, and safety and immune responses were assessed. CVD 908-**htrA** and CVD 906-**htrA** were well tolerated in volunteers; mild diarrhea in 3 of 36 volunteers and mild fever in 1 volunteer were the only notable adverse responses. The **vaccine** strains were not detected in blood cultures and only transiently detected in stool. Serum immune responses to S. typhi lipopolysaccharide and H antigens were observed in 75 to 100% of volunteers who received 5 x 10(8) to 5 x 10(9) CFU, and cells secreting S. typhi-specific antibodies were found in all volunteers after ingestion of either strain. Sixty-three percent to 83% of volunteers developed lymphoproliferative responses to S. typhi flagellar and particulate antigens after the higher doses. These studies demonstrate the potential of CVD 908-**htrA** as a live vector for the delivery of heterologous genes, and a clinical trial of such a construct is planned.

L61 ANSWER 15 OF 42 CAPLUS COPYRIGHT 2001 ACS
1997:358711 Document No. 127:120395 Attenuated **Salmonella** typhi and Shigella as live oral **vaccines** and as live vectors. Levine, M. M.; Galen, J.; Barry, E.; Noriega, F.; Tacket, C.; Sztein, M.; **Chatfield, S.**; Dougan, G.; Losonsky, G.; Kotloff, K. (School Medicine, Univ. Maryland, Baltimore, MD, 21201, USA). Behring Inst. Mitt., 98(New Approaches to Bacterial Vaccine Development), 120-123 (English) 1997. CODEN: BHIMA2. ISSN: 0301-0457. Publisher: Medizinische Verlagsgesellschaft mbH.

AB A review is given with 26 refs. including the authors own works on new generations of attenuated **Salmonella** typhi and Shigella strains with precise, defined mutations for use as live oral **vaccines** and on the live vectors CVD 908 and CVD 908-**htrA**.

DUPLICATE 10

L61 ANSWER 16 OF 42 MEDLINE
96376098 Document Number: 96376098. PubMed ID: 8782354. Immunisation of mice using **Salmonella** typhimurium expressing human papillomavirus type 16 E7 epitopes inserted into hepatitis B virus core antigen. Londono L P; **Chatfield S**; Tindle R W; Herd K; Gao X M; Frazer I; Dougan G. (Department of Biochemistry, Imperial College of Science, Technology and Medicine, London, UK.) VACCINE, (1996 Apr) 14 (6) 545-52. Journal code: X6O; 8406899. ISSN: 0264-410X. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Live **vaccines** based on BRD509, an attenuated S. typhimurium (**aroA**, **aroD**) strain, were constructed that directed the expression of hepatitis B core antigen particles (HBcAg) (BRD969) or HBcAg harbouring human papillomavirus type 16 E7 protein sequences (BRD974), under the control of the in vivo inducible nirB promoter. These strains were used to orally or intravenously immunise different inbred mouse strains and humoral, secretory and cellular anti-E7 and anti-HBcAg responses were monitored. Both BRD969 and BRD974 induced anti-HBcAg humoral IgG responses following oral or intravenous immunisation of B10 mice, although responses were higher in BRD969 immunised animals. IgG subclass analysis revealed a predominantly IgG2a response in these

animals. BRD974, but not BRD969, induced anti-E7 humoral IgG responses. Anti-HBcAg (BRD969 and BRD974) and anti-E7 (BRD974) IgA responses were detected in the intestines of orally immunised mice. Anti-**Salmonella** but not anti-HBcAg or anti-E7 T helper cell responses were detected in mice immunised with BRD509, BRD969 and BRD974. Thus **Salmonella vaccine** strains can be used to efficiently deliver HBcAg and E7 epitopes to the mucosal and systemic immune systems.

L61 ANSWER 17 OF 42 MEDLINE DUPLICATE 11
96351471 Document Number: 96351471. PubMed ID: 8717403. Attenuated **Salmonella** as live oral **vaccines** against typhoid fever and as live vectors. Levine M M; Galen J; Barry E; Noriega F; **Chatfield S**; Sztein M; Dougan G; Tacket C. (Center for Vaccine Development, University of Maryland School of Medicine, Baltimore 21201, USA.) JOURNAL OF BIOTECHNOLOGY, (1996 Jan 26) 44 (1-3) 193-6. Ref: 19. Journal code: AL6; 8411927. ISSN: 0168-1656. Pub. country: Netherlands. Language: English.

AB Attenuated **Salmonella** typhi **vaccine** strain CVD 908, which harbors deletion mutations in **aroC** and **aroD**, has been shown to be well-tolerated and highly immunogenic, eliciting impressive serum antibody, mucosal IgA and cell-mediated immune responses.

A further derivative prepared by introducing a deletion in **htrA** (which encodes a heat-shock protein that also has activity as a serine protease in CVD 908 (Chatfield et al., unpublished data) resulted in CVD 908-**htrA**. In phase 1 clinical trials, CVD 908-**htrA** appears very attractive as a live oral **vaccine** candidate. Both CVD 908 and CVD 908-**htrA** are useful as live vector **vaccines** to deliver foreign antigens to the immune system. Conditions that enhance the expression and immunogenicity of foreign antigens carried by CVD 908 and CVD 908-**htrA** are being investigated.

L61 ANSWER 18 OF 42 CAPLUS COPYRIGHT 2001 ACS
1995:890197 Document No. 123:278079 Use of the promoter of the heat-shock gene **htrA** for expression of antigen genes in **vaccine** strains of bacteria. Khan, Mohammed Anjam; **Chatfield, Steven Neville**; Li, Jingli (Medeva Holdings B.V., Neth.). PCT Int. Appl. WO 9520665 A1 19950803, 54 pp. DESIGNATED STATES: W: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, US; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1995-GB196 19950131. PRIORITY: GB 1994-1795 19940131.

AB The promoter of the **htrA** gene is used to express foreign genes in attenuated **vaccine** strains of bacteria, esp. of **Salmonella**. This promoter gives higher levels of expression than the nirB or groE promoters. These expression constructs can be used to prep. a **vaccine** strain that presents antigens assocd. with other diseases as a multivalent live **vaccine**. Fusion proteins of antigens in which the sep. domains are connected by a flexible hinge peptide such as that from Igs are described. Expression vectors using the **htrA** promoter to drive expression of the tetanus toxin C fragment

gene were prepd. One of these vectors was modified to simplify the introduction of a coding fragment 3' to the toxin coding sequence for manuf. of fusion proteins. A reporter gene under control of this promoter was induced by temp. shifts in macrophage cell lines infected with bacteria carrying it. Expression was further induced by exposure to hydrogen peroxide levels found in macrophages.

L61 ANSWER 19 OF 42 MEDLINE DUPLICATE 12
96071868 Document Number: 96071868. PubMed ID: 7591105. Differential induction of carrier antigen-specific immunity by **Salmonella** typhimurium live-**vaccine** strains after single mucosal or intravenous immunization of BALB/c mice. Karem K L; **Chatfield S**; Kuklin N; Rouse B T. (Department of Microbiology, University of Tennessee College of Veterinary Medicine, Knoxville 37996, USA.) INFECTION AND IMMUNITY, (1995 Dec) 63 (12) 4557-63. Journal code: GO7; 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB In this study, we constructed strain KR21 (chi 4550 delta **cya** delta **crp** delta asd/pYA292asd(+)-toxC+) and compared it with BRD847 (**aroA aroD**/pnirB-toxC) for the ability to induce humoral and cellular immunity after a single oral or intravenous immunization in 3- to 4-week-old BALB/c mice. ToxC-specific serum immunoglobulin G (IgG) was detectable in animals orally immunized with either BRD847 or KR21. However, after intravenous immunization, IgG was detected only in BRD847-immunized animals. Measurement of immunoglobin types IgG1 and IgG2a suggests that a Th1 cellular response is prominent after immunizations with either system. ToxC-specific IgA was detected in fecal and vaginal samples of animals immunized orally and intravenously with BRD847, while those immunized with KR21 failed to show fecal or vaginal IgA responses. Delayed-type hypersensitivity was used as a measure of induction of T-cell responses in vivo. Mice immunized either orally or intravenously with BRD847 showed significant ear swelling responses after ToxC injections, while KR21-immunized animals failed to show a cellular response. These data indicate that the **aroA aroD**/pnirB system holds greater potential for inducing global immunity after a single dose when directly compared with the balanced lethal system (delta **cya** delta **crp** delta asd/pYA292asd+).

L61 ANSWER 20 OF 42 MEDLINE DUPLICATE 13
95310012 Document Number: 95310012. PubMed ID: 7790070. Influence of preimmunization with tetanus toxoid on immune responses to tetanus toxin fragment C-guest antigen fusions in a **Salmonella vaccine** carrier. Chabalgoity J A; Villareal-Ramos B; Khan C M; **Chatfield S N**; de Hormaeche R D; Hormaeche C E. (Department of Microbiology, Medical School, University of Newcastle, Newcastle-upon-Tyne, United Kingdom.) INFECTION AND IMMUNITY, (1995 Jul) 63 (7) 2564-9. Journal code: GO7; 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB We have previously described a new system for the delivery of recombinant antigens in live **Salmonella vaccines** as genetic fusions to the C terminus of fragment C of tetanus toxin (TetC) driven by the anaerobically inducible nirB promoter. It has been reported that preimmunization with tetanus toxoid (TT) can suppress the antibody

response to peptides chemically coupled to TT (epitope-specific suppression) in both animals and humans, which could interfere with efficacy of the **Salmonella**-TetC delivery system. We report that preimmunization of BALB/c mice with TT in alum did not suppress the response to either of two protective antigens of Schistosoma mansoni, the full-length S. mansoni P28 glutathione S-transferase (P28) and a construct consisting of eight tandem copies of the protective peptide comprising amino acids 115 to 131 of P28. The guest antigens were expressed in the **aroA Salmonella** typhimurium SL3261 **vaccine** strain as fusions to TetC. Preimmunization with TT 10 weeks before administration of the recombinant **salmonellae** did not alter the antibody response to the full-length P28, whereas the response to the peptide comprising amino acids 115 to 131 was increased by preimmunization with TT, with the increase seen mainly in the immunoglobulin G1 isotype. The antitetanus response was increased by preimmunization with TT in all groups receiving **salmonellae** expressing TetC. The results could be important when one is considering the use of the **Salmonella** -TetC delivery system in populations preimmunized with TT.

L61 ANSWER 21 OF 42 MEDLINE DUPLICATE 14

95203672 Document Number: 95203672. PubMed ID: 7896085. Expression of LacZ from the **htrA**, nirB and groE promoters in a **Salmonella vaccine** strain: influence of growth in mammalian cells. Everest P; Frankel G; Li J; Lund P; **Chatfield S** ; Dougan G. (Department of Biochemistry, Imperial College of Science, Technology and Medicine, London, UK.) FEMS MICROBIOLOGY LETTERS, (1995 Feb 1) 126 (1) 97-101. Journal code: FML; 7705721. ISSN: 0378-1097. Pub. country: Netherlands. Language: English.

AB Attenuated **Salmonella** strains are currently being evaluated as live vectors for the delivery of heterologous antigens to the mammalian mucosal and systemic immune systems. An approach to improving the stability of heterologous antigen expression during **vaccination** is to drive expression of the foreign protein from promoters, e.g. nirB, that become activated when **Salmonella** enter the host. **Salmonella** strains were constructed that harboured similar multicopy plasmids encoding the lacZ gene. In each strain, lacZ expression was driven from either the nirB, **htrA** or groE promoters. Expression of LacZ increased in all **vaccine** strains as they were shifted from conditions of low to high temperature. In addition, expression of lacZ driven from the **htrA** and nirB promoters significantly increased when the **Salmonella** entered eukaryotic cells, including macrophages. Expression of lacZ from the groE promoter was significantly elevated in macrophages but not in cells derived from epithelia. These promoters may be useful for optimising heterologous antigen expression within immune cells of the host.

L61 ANSWER 22 OF 42 MEDLINE DUPLICATE 15

95362289 Document Number: 95362289. PubMed ID: 7635511. Protection against Leishmania major infection in genetically susceptible BALB/c mice by gp63 delivered orally in attenuated **Salmonella** typhimurium (**AroA- AroD-**). Xu D; McSorley S J; **Chatfield S N** ; Dougan G; Liew F Y. (Department of Immunology, University of Glasgow,

UK.) IMMUNOLOGY, (1995 May) 85 (1) 1-7. Journal code: GH7; 0374672. ISSN: 0019-2805. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The gene encoding the Leishmania major (L. major) promastigote surface glycoprotein, gp63, was introduced into the **Salmonella** typhimurium (S. typhimurium) **aroA**- **aroD**- live oral **vaccine** strain BRD509 and expressed under the control of a constitutive tac promoter in plasmid pKK233-2. This construct (GID101) expressed gp63 in vitro and was used to immunize highly susceptible BALB/c mice by the oral route. The plasmid was relatively stably inherited by bacteria growing or persisting in the mesenteric lymph nodes of immunized mice. Mice immunized with GID101 developed significant resistance against a challenge infection with L. major compared to controls immunized with BRD509 alone. Spleen and lymph node cells from immunized mice developed a strong in vitro proliferative T-cell response to killed or live L. major. The activated T cells secreted interleukin-2 (IL-2) and interferon-gamma (IFN-gamma) which was abrogated by treatment with anti-CD4 but not with anti-CD8 antibody. The cells did not produce detectable levels of interleukin-4 (IL-4). The immunized mice also produced significant amounts of leishmanial specific IgG2a antibody but did not develop delayed-type hypersensitivity (DTH) to live parasites. No IgG1 antibody was detected. These data therefore demonstrate that gp63 gene delivered orally by a **vaccine** strain of S. typhimurium can preferentially induce the development of Th-1 subset of CD4+ T cells and protective immunity in the highly susceptible BALB/c mice.

L61 ANSWER 23 OF 42 MEDLINE DUPLICATE 16

95062246 Document Number: 95062246. PubMed ID: 7972044. Construction, expression, and immunogenicity of the Schistosoma mansoni P28 glutathione S-transferase as a genetic fusion to tetanus toxin fragment C in a live Aro attenuated **vaccine** strain of **Salmonella**. Khan C M; Villarreal-Ramos B; Pierce R J; Riveau G; Demarco de Hormaeche R; McNeill H; Ali T; Fairweather N; **Chatfield S**; Capron A; +. (Department of Pathology, University of Cambridge, United Kingdom.) PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1994 Nov 8) 91 (23) 11261-5. Journal code: PV3; 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB A vector has been constructed to allow genetic fusions of guest antigens via a hinge domain to the C terminus of the highly immunogenic C fragment of tetanus toxin. A fusion has been constructed with the gene encoding the protective 28-kDa glutathione S-transferase (EC 2.5.1.18) from Schistosoma mansoni. The recombinant vector has been electroporated into the nonvirulent **Salmonella** typhimurium **aroA** live **vaccine** strain SL3261. The corresponding chimeric protein is stably expressed in a soluble form in **Salmonella** as evaluated by Western blotting with fragment C and glutathione S-transferase antisera. Mice immunized intravenously with a single dose of the live recombinant bacteria elicit antibodies to both fragment C and glutathione S-transferase as detected by enzyme-linked immunosorbent assays. Furthermore, all of the mice were solidly protected when challenged with

lethal doses of either tetanus toxin or the virulent **Salmonella** typhimurium strain C5. Mice have also elicited antibodies to fragment C and glutathione S-transferase after oral immunization. It may be that a live trivalent **vaccine** against typhoid, tetanus, and schistosomiasis is feasible.

DUPLICATE 17

L61 ANSWER 24 OF 42 MEDLINE
95081611 Document Number: 95081611. PubMed ID: 7527446. Construction, expression, and immunogenicity of multiple tandem copies of the Schistosoma mansoni peptide 115-131 of the P28 glutathione S-transferase expressed as C-terminal fusions to tetanus toxin fragment C in a live aro-attenuated **vaccine** strain of **Salmonella**. Khan C M; Villarreal-Ramos B; Pierce R J; Demarco de Hormaeche R; McNeill H; Ali T; **Chatfield S**; Capron A; Dougan G; Hormaeche C E. (Department of Pathology, University of Cambridge, UK.) JOURNAL OF IMMUNOLOGY, (1994 Dec 15) 153 (12) 5634-42. Journal code: IFB; 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB Genetic fusions have been constructed between the highly immunogenic but atoxic fragment C of tetanus toxin and a guest peptide, aa115-131, from the protective 28-kDa glutathione S-transferase Ag of Schistosoma mansoni.
Fusions have been assembled to express one, two, four, and eight tandem copies of the peptide. The recombinant vectors have been electroporated into the nonvirulent **aroA** strain of **Salmonella** typhimurium SL3261. The fusion proteins are soluble and stably expressed in **Salmonella** as evaluated by Western blotting with fragment C and glutathione S-transferase antisera. Mice have been immunized i.v. with a single dose of the live recombinant **salmonellae**. The strains are stable in mice and elicit Ab responses directed against fragment C, as determined by enzyme-linked immunosorbent assays. Ab responses were also detected against the guest peptide. The Ab responses improved dramatically toward the aa115-131 peptide with increasing copy number, with the octameric "repitope" fusion displaying the greatest potency. This approach may represent a general strategy for eliciting immune responses against peptides in live bacterial **vaccines**.

L61 ANSWER 25 OF 42 MEDLINE
94341908 Document Number: 94341908. PubMed ID: 8063417. Characterization of defined ompR mutants of **Salmonella** typhi: ompR is involved in the regulation of Vi polysaccharide expression. Pickard D; Li J; Roberts M; Maskell D; Hone D; Levine M; Dougan G; **Chatfield S**. (Department of Biochemistry, Imperial College of Science, Technology and Medicine, London, United Kingdom.) INFECTION AND IMMUNITY, (1994 Sep) 62 (9) 3984-93. Journal code: GO7; 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB The ompB operon, comprising the ompR and envZ genes, was cloned from a **Salmonella** typhi Ty2 cosmid bank and characterized by DNA sequence analysis. The S. typhi ompR and envZ genes contained open reading frames encoding proteins of 240 and 451 amino acids, respectively. Comparison with the **Salmonella** typhimurium OmpB protein sequences revealed

99.5% homology. The DNA sequence data were used to identify appropriate restriction sites for generating a defined deletion of 517 bp within the open reading frame of the ompR gene. This deletion was introduced by homologous recombination into the chromosomes of two S. typhi strains which already harbored defined deletions in both the **aroC** and **aroD** genes. The presence of the deletions within ompR was confirmed by Southern hybridization and sequencing of the DNA fragments surrounding the deleted regions by PCR. The S. typhi ompR mutants displayed a marked decrease in **OmpC** and **OmpF** porin expression as demonstrated by examination of outer membrane preparations. It was also found that S. typhi strains harboring the defined ompR deletions no longer agglutinated with Vi antiserum. However, when a functional ompB operon was introduced back into the S. typhi ompR mutants, either on a multicopy plasmid or as a single-copy chromosomal replacement, the Vi+ phenotype was restored. The levels of Vi synthesis were also found to be sensitive to different concentrations of sodium chloride present in the growth medium, although the levels of sensitivity varied between different isolates of S. typhi. It is therefore concluded that the ompR-envZ two component regulatory system plays an important role in the regulation of Vi polysaccharide synthesis in S. typhi and that one of the environmental signals for this regulation may be osmolarity.

L61 ANSWER 26 OF 42 MEDLINE
95046941 Document Number: 95046941. PubMed ID: 7958481. The use of live attenuated **Salmonella** for oral **vaccination**.
Chatfield S; Roberts M; Li J; Starns A; Dougan G. (Medeva Vaccine Research Unit, Imperial College of Science, Technology and Medicine, London, UK.) DEVELOPMENTS IN BIOLOGICAL STANDARDIZATION, (1994) 82 35-42.
Ref: 23. Journal code: E7V; 0427140. ISSN: 0301-5149. Pub. country: Switzerland. Language: English.

AB Studies of the pathogenesis of **Salmonella** at the molecular level have led to the identification of several classes of genes that are involved in survival in the host. This has led to the availability of a panel of attenuating lesions which are now being used to develop several rationally attenuated strains which are being evaluated as oral **vaccines** against human and animal salmonellosis. Much effort has been directed towards the development of a more efficacious single dose oral typhoid **vaccine** and there are now several candidates in Phase 1 studies. The successful development of a genetically defined oral typhoid **vaccine** will not only be a major step forward in the control of typhoid but will pave the way for development of practical human **vaccines** based on using the strain to deliver heterologous antigens to the human immune system. We have concentrated on developing a single dose oral tetanus **vaccine** based on constructing strains expressing fragment C (a non-toxic immunogenic protein derived from tetanus toxin). Several different promotors have been used for controlling the expression of fragment C and these have been introduced into double aro mutants of S. typhimurium and compared for their ability to elicit protective immune responses in mice. This work has demonstrated that it is

possible to protect mice against tetanus toxin challenge after a single oral dose of one of these recombinant **Salmonella** strains. Analogous hybrid S. typhi double aro mutants have now been constructed for potential use in humans.

L61 ANSWER 27 OF 42 SCISEARCH COPYRIGHT 2001 ISI (R)
93:388120 The Genuine Article (R) Number: LH325. PROTECTION OF MICE AGAINST RESPIRATORY **BORDETELLA**-PERTUSSIS INFECTION BY INTRANASAL IMMUNIZATION WITH P.69 AND FHA. ROBERTS M (Reprint); CROPLEY I; **CHATFIELD S**; DOUGAN G. UNIV LONDON IMPERIAL COLL SCI TECHNOL & MED, MEDEVA GRP RES, VACCINE RES UNIT, LONDON SW7 2AY, ENGLAND (Reprint); UNIV LONDON IMPERIAL COLL SCI TECHNOL & MED, DEPT BIOCHEM, LONDON SW7 2AY, ENGLAND. VACCINE (JUN 1993) Vol. 11, No. 8, pp. 866-872. ISSN: 0264-410X.
Pub. country: ENGLAND. Language: ENGLISH.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB Intranasal immunization of adult female Balb/c mice with the **Bordetella** pertussis antigens FHA or P.69, greatly enhanced their ability to clear B. pertussis from their lungs following aerosol challenge compared with ovalbumin-immunized controls. Low numbers of lymphocytes secreting antibodies (IgG, IgA and IgM) against the immunizing antigens could be isolated from the lungs of immunized mice. Following aerosol challenge with B. pertussis there was a large increase in the numbers of FHA or P.69-specific antibody-secreting cells in the lungs of mice immunized with these antigens. Intranasal immunization, particularly with FHA, also primed mice to develop a systemic serum anti-pertussis antibody response subsequent to challenge. However, pulmonary clearance of B. pertussis correlated most closely with the local antibody response. A strong anti-FHA response was demonstrated in the lungs of mice that received a booster dose of FHA 9 months after their previous exposure to FHA, demonstrating that long immunological memory can develop in the murine respiratory tract following direct application of pertussis antigens to the respiratory tract mucosa.

L61 ANSWER 28 OF 42 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 18
1993:109672 Document No. 118:109672 Attenuated bacteria expressing antigenic protein genes and their use as **vaccines**. Charles, Ian George; **Chatfield, Steven Neville**; Fairweather, Neil Fraser (Wellcome Foundation Ltd., UK). PCT Int. Appl. WO 9215689 A1 19920917, 23 pp. DESIGNATED STATES: W: AT, AU, BB, BG, BR, CA, CH, CS, DE, DK, ES, FI, GB, HU, JP, KP, KR, LK, LU, MG, MN, MW, NL, NO, PL, RO, RU, SD, SE, US; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GR, IT, LU, MC, ML, MR, NL, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1992-GB387 19920305. PRIORITY: GB 1991-4596 19910305; GB 1991-21208 19911004.
AB Attenuated bacteria contg. an antigenic protein gene fused to a promoter whose activity is induced by anaerobic conditions are described. These transformants can be used as **vaccines**. **Salmonella** typhimurium (**aroA-aroD**-) were transformed with a plasmid contg. the gene for tetanus toxin fragment C fused to the nirB

promoter of **Escherichia** coli. These bacteria were effective single-dose oral **vaccines** against tetanus toxin challenge in mice.

DUPLICATE 19

L61 ANSWER 29 OF 42 MEDLINE
93014094 Document Number: 93014094. PubMed ID: 1398911. Characterization of a **Salmonella** typhimurium aro **vaccine** strain expressing the P.69 antigen of **Bordetella** pertussis. Strugnell R; Dougan G; **Chatfield S**; Charles I; Fairweather N; Tite J; Li J L; Beesley J; Roberts M. (Department of Cell Biology, Wellcome Research Laboratories, Langley Court, Beckenham, Kent, United Kingdom.) INFECTION AND IMMUNITY, (1992 Oct) 60 (10) 3994-4002. Journal code: GO7; 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB The P.69 **Bordetella** pertussis protective antigen was expressed by use of the trc promoter from the chromosome of a **Salmonella** typhimurium aro **vaccine** strain, BRD509, by integrating the prn gene, encoding the 93-kDa precursor of this protein, into the **aroC** locus. P.69 was detected on the cell surface of the S. typhimurium strain (BRD640) by agglutination and immunoelectron microscopy. BALB/c mice immunized orally or intravenously with BRD640 showed a significant level of protection against an aerosol challenge with virulent B. pertussis, compared with control animals. No anti-P.69 antibodies in the serum or anti-P.69 antibody-secreting cells in the lungs were detected in BRD640-**vaccinated** animals, although cells isolated from spleens showed a P.69-dependent cell proliferative response. In contrast, low levels of anti-P.69 antibodies in the serum and anti-P.69 antibody-secreting cells in the lungs were detected in immunized mice following a B. pertussis challenge.

DUPLICATE 20

L61 ANSWER 30 OF 42 MEDLINE
92148139 Document Number: 92148139. PubMed ID: 1737934. Expression of human IL-1 beta in **Salmonella** typhimurium. A model system for the delivery of recombinant therapeutic proteins in vivo. Carrier M J; **Chatfield S N**; Dougan G; Nowicka U T; O'Callaghan D; Beesley J E; Milano S; Cillari E; Liew F Y. (Wellcome Research Laboratories, Beckenham, Kent, England.) JOURNAL OF IMMUNOLOGY, (1992 Feb 15) 148 (4) 1176-81. Journal code: IFB; 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB The feasibility of using **Salmonella** typhimurium **aroA** mutant (SL3261) to deliver protein therapeutic agents was investigated in a murine model system. We have constructed an **Escherichia** coli expression plasmid designed to express the human protein IL-1 beta. This plasmid expresses IL-1 beta to high levels (greater than 30% total cell protein) in E. coli. In **Salmonella** the IL-1 beta is expressed constitutively to about 10% total cell protein, as verified by Western blotting analysis using polyclonal rabbit anti-IL-1 beta antibody. The protein is produced in a soluble and biologically active form. BALB/c mice administered orally or i.v. with S. typhimurium **aroA** mutants carrying the plasmid produced highly significant antibody responses against human IL-1 beta as determined by a solid-phase RIA. Furthermore, mice injected with the construct were significantly protected against lethal gamma-irradiation (850 rad). This study therefore demonstrates that

the **vaccine** strain of **Salmonella** mutants can also be used effectively to deliver therapeutic proteins in vivo.

DUPLICATE 21

L61 ANSWER 31 OF 42 MEDLINE
93080910 Document Number: 93080910. PubMed ID: 1368983. Use of the nirB promoter to direct the stable expression of heterologous antigens in **Salmonella** oral **vaccine** strains: development of a single-dose oral tetanus **vaccine**. **Chatfield S N**; Charles I G; Makoff A J; Oxer M D; Dougan G; Pickard D; Slater D; Fairweather N F. (Department of Biochemistry, Imperial College of Science, Technology and Medicine, London, UK.) BIO/TECHNOLOGY, (1992 Aug) 10 (8) 888-92. Journal code: AL1; 8309273. ISSN: 0733-222X. Pub. country: United States. Language: English.

AB Plasmid pTETnir15, which directs the expression of the non-toxic immunogenic fragment C of tetanus toxin from the anaerobically inducible nirB promoter, was introduced into the **Salmonella** typhimurium **aroA aroD** live oral **vaccine** strain BRD509. The resulting strain, designated BRD847, was used to **vaccinate** orally BALB/c mice and was tested for plasmid stability and its ability to protect against a lethal tetanus toxin challenge. pTETnir15 was stably inherited by bacteria growing or persisting in the tissues of immunized mice whereas another BRD509 derivative, designated BRD753, harboring plasmid pTET85 which directs fragment C expression from the tac promoter, was highly unstable. Mice immunized with a single oral dose of BRD847 developed high levels of circulating anti-fragment C antibodies and were solidly protected against tetanus toxin challenge. Mice immunized with a single oral dose of BRD753 developed no detectable anti-fragment C antibodies. After boosting, antibodies were detected, but the mice were only partially protected against tetanus toxin challenge. Thus the use of an in vivo inducible promoter such as nirB may be a generally applicable approach to obtaining the stable in vivo expression of heterologous antigens in **Salmonella vaccine** strains.

DUPLICATE 22

L61 ANSWER 32 OF 42 MEDLINE
92334130 Document Number: 92334130. PubMed ID: 1630300. Impaired resistance to infection does not increase the virulence of **Salmonella htrA** live **vaccines** for mice.
Strahan K; **Chatfield S N**; Tite J; Dougan G; Hormaeche C E. (Department of Pathology, Cambridge, U.K.) MICROBIAL PATHOGENESIS, (1992 Apr) 12 (4) 311-7. Journal code: MIC; 8606191. ISSN: 0882-4010. Pub. country: ENGLAND: United Kingdom. Language: English.

AB We have described a new class of live attenuated **salmonella vaccines** harbouring lesions in **htrA**, a stress protein gene previously. The virulence and invasiveness of **Salmonella htrA** mutants was investigated in three models of increased susceptibility to **Salmonella** infection. These included BALB/c mice, either given sublethal whole body irradiation (350 R) or administered rabbit anti-TNF alpha antiserum, and (CBA/NfemaleXBALB/cmale)F1 male mice which express the xid sex-linked B cell defect of CBA/N mice and are more susceptible to **salmonellae** than female littermates. **Salmonella** typhimurium **htrA** mutants derived from virulent strains, C5046 (C5 **htrA**::TnphoA)

and BRD726 (SL1344 delta **htrA**) were not more invasive in immunosuppressed mice than in normal controls in the three mouse models of defective immunity. The results indicate that susceptibility to S. typhimurium **htrA vaccines** derived from virulent parents is not enhanced by conditions of impaired resistance to infection.

L61 ANSWER 33 OF 42 MEDLINE DUPLICATE 23
92261298 Document Number: 92261298. PubMed ID: 1584006. Evaluation of **Salmonella** typhimurium strains harbouring defined mutations in **htrA** and **aroA** in the murine salmonellosis model. **Chatfield S N**; Strahan K; Pickard D; Charles I G; Hormaeche C E; Dougan G. (Vaccines Research Unit, Medeva Group Research, Wellcome Research Labs, Beckenham, Kent, U.K.) MICROBIAL PATHOGENESIS, (1992 Feb) 12 (2) 145-51. Journal code: MIC; 8606191. ISSN: 0882-4010. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Derivatives of the mouse-virulent **Salmonella** typhimurium strain SL1344 were constructed harbouring defined mutations in **htrA**, **aroA** or **htrA aroA** combined. When administered orally or intravenously to BALB/c mice, all the mutants were found to be highly attenuated. All mutants were able to confer significant protection against lethal challenge with SL1344 after a single oral dose of live organisms. SL1344 **htrA** mutants persisted in livers and spleens at a lower level than SL1344 **aroA** mutants after intravenous administration. SL1344 **htrA aroA** mutants persisted at an even lower level and were cleared from the livers and spleens of mice within 21 days of intravenous administration. Thus **htrA** and **htrA aroA** mutants can be considered as potential oral **vaccines** against salmonellosis.

L61 ANSWER 34 OF 42 MEDLINE DUPLICATE 24
92170207 Document Number: 92170207. PubMed ID: 1311488. Construction of a genetically defined **Salmonella** typhi Ty2 **aroA**, **aroC** mutant for the engineering of a candidate oral typhoid-tetanus **vaccine**. **Chatfield S N**; Fairweather N; Charles I; Pickard D; Levine M; Hone D; Posada M; Strugnell R A; Dougan G. (Vaccine Research Unit, Wellcome Research Labs, Beckenham, Kent, UK.) VACCINE, (1992) 10 (1) 53-60. Journal code: X60; 8406899. ISSN: 0264-410X. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The construction of a **Salmonella** typhi Ty2 strain harbouring defined deletions in both the **aroA** and **aroC** genes is described. These deletions have been fully defined at the molecular level by DNA sequencing and have been introduced in such a way that no foreign DNA remains in the S. typhi genome. This strain is attenuated in mice when given by the intraperitoneal route suspended in hog gastric mucin and is attenuated to a similar level to strains harbouring deletions in **aroA** or **aroC** alone indicating that both lesions are capable of attenuating independently. We have used this defined S. typhi **aroA aroC** strain to express stably a non-toxic 50 kDa fragment of tetanus toxin (fragment C) from a gene incorporated into the

chromosome. This strain has the advantage of harbouring no antibiotic-resistance markers and we consider it to be a candidate bivalent oral typhoid-tetanus **vaccine**.

L61 ANSWER 35 OF 42 MEDLINE DUPLICATE 25
92013148 Document Number: 92013148. PubMed ID: 1919009. The involvement of tumor necrosis factor in immunity to **Salmonella** infection. Tite J P; Dougan G; **Chatfield S N**. (Department of Molecular Biology, Wellcome Biotech, Beckenham, Kent, UK.) JOURNAL OF IMMUNOLOGY, (1991 Nov 1) 147 (9) 3161-4. Journal code: IFB; 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.
AB The role of TNF in immunity to **Salmonella** in mice was studied. Antiserum specific for murine TNF was raised and used to neutralize TNF activity in vivo. Injection of this serum into mice infected with the moderately mouse virulent **Salmonella** typhimurium strain M525 caused exacerbation of disease. Such treatment had no effect on the course of an infection with an attenuated S. typhimurium **aroA** (strain SL3261) mutant. However, the protection afforded by immunisation with live SL3261 against challenge with the virulent parent strain (SL1344) was abolished by anti-TNF antiserum. Interestingly both early (3 wk) immunity and late (10 wk) immunity was neutralized by such treatment. Inasmuch as early immunity is considered to be nonspecific and macrophage-mediated while late immunity is considered to be serotype-specific and T cell mediated, this suggests that TNF plays a role in protection from Salmonellosis in both cases.

L61 ANSWER 36 OF 42 MEDLINE DUPLICATE 26
92101612 Document Number: 92101612. PubMed ID: 1759503. Construction of genetically defined double aro mutants of **Salmonella** typhi. Hone D M; Harris A M; **Chatfield S**; Dougan G; Levine M M. (Department of Medicine, University of Maryland School of Medicine, University of Maryland, Baltimore 21201.) VACCINE, (1991 Nov) 9 (11) 810-6. Journal code: X60; 8406899. ISSN: 0264-410X. Pub. country: ENGLAND: United Kingdom. Language: English.
AB The construction of genetically defined, double aro mutant strains CVD906 and CVD908, which were derived from **Salmonella** typhi strain ISP1820 (a recent isolate of S. typhi from Chile) and from laboratory strain Ty2, respectively, is described. Strains CVD906 and CVD908 differ from previously described aro mutants of S. typhi as their aro deletion mutations do not extend beyond the limits of the mutated aro genes, and no antibiotic-resistance genes, plasmid sequences or S. typhimurium DNA sequences remain in the mutant strains. In minimal medium the aro mutants of S. typhi are unable to replicate whereas the wild type parent strains grow well in minimal medium. Using intraperitoneal inoculation of mice with S. typhi strains suspended in hog gastric mucin as a virulence assay, it is shown that the single aro mutants and the double aro mutants of Ty2 and ISP1820 are attenuated in mice. Trans complementation of the aro mutants with the **aroC** gene or **aroD** gene, or both, results in strains that are phenotypically identical to that of the wild type parents indicating that no measurable additional changes other than loss of the aro gene function occurred during strain construction.

this is the first example of a successful oral **vaccination** that uses an attenuated bacterial carrier to deliver a protective antigen derived from tetanus toxin.

L61 ANSWER 39 OF 42 CAPLUS COPYRIGHT 2001 ACS
1990:84145 Document No. 112:84145 Live **vaccines** containing attenuated microorganisms having double mutations in genes in the aromatic
biosynthetic pathway. Dougan, Gordon; **Chatfield, Steven Neville** (Wellcome Foundation Ltd., UK). Eur. Pat. Appl. EP 322237 A1 19890628, 13
pp. DESIGNATED STATES: R: AT, BE, CH, DE, ES, FR, GB, GR, IT, LI, LU, NL, SE. (English). CODEN: EPXXDW. APPLICATION: EP 1988-312203 19881222.
PRIORITY: GB 1987-30037 19871223.

AB An attenuated microorganism harboring 2 mutated genes, each of which is located in the organism's arom. biosynthetic pathway is useful as a **vaccine**. The attenuated microorganism can be genetically engineered so as to express antigens from other pathogens, thus making a range of multivalent **vaccines**. **Salmonella** typhimurium **aroA aroC** double mutant was prepd. by transposon mutagenesis. Balb/c mice treated by oral administration of 109-1010 of the mutant resisted oral challenge by the parental virulent strain (SL 1344) of S. typhimurium 28 and 70 days post immunization. Oral tablets contained freeze-dried S. typhi double mutant 70.0, Aerosil-200 0.5, Dipac
235.0, crosslinked Povidone 7.0, microcryst. cellulose, 35.0, and Mg stearate 2.5 mg coated with Opadry Enteric OY-P-7156 35.0 mg.

DUPLICATE 29

L61 ANSWER 40 OF 42 MEDLINE
89277533 Document Number: 89277533. PubMed ID: 2543631. Characterization of porin and ompR mutants of a virulent strain of **Salmonella** typhimurium: ompR mutants are attenuated in vivo. Dorman C J; **Chatfield S**; Higgins C F; Hayward C; Dougan G. (Department of Biochemistry, University of Dundee, United Kingdom.) INFECTION AND IMMUNITY, (1989 Jul) 57 (7) 2136-40. Journal code: GO7; 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB The **ompC**, ompD, and **ompF** genes encode the three major porins of **Salmonella** typhimurium. ompR encodes a positive regulator required for the expression of **ompC** and **ompF** . Transposon-generated mutations in **ompC**, ompD, **ompF**, and ompR were introduced into the S. typhimurium mouse virulent strain SL1344 by P22-mediated transduction. Following preliminary characterization in vitro, the strains were used to challenge BALB/c mice by using the oral or intravenous route. Strains harboring **ompC** or **ompF** mutations were as virulent as SL1344 after oral challenge. Strains harboring ompD mutations had a slight reduction in virulence. In contrast, ompR mutants failed to kill BALB/c mice after oral
challenge and the intravenous 50% lethal dose was reduced by approximately
10(5). The ompR mutants persisted in murine tissues for several weeks following oral or intravenous challenge. Furthermore, mice orally immunized with these ompR mutant strains were well protected against challenge with virulent SL1344.

DUPLICATE 30

L61 ANSWER 41 OF 42 MEDLINE
89218937 Document Number: 89218937. PubMed ID: 2523513. Bacteriophage P22
as a vehicle for transducing cosmid gene banks between smooth strains of **Salmonella** typhimurium: use in identifying a role for **aroD** in attenuating virulent **Salmonella** strains. Miller I A; **Chatfield S**; Dougan G; Desilva L; Joysey H S; Hormaeche C. (Department of Pathology, University of Cambridge, UK.) MOLECULAR AND GENERAL GENETICS, (1989 Jan) 215 (2) 312-6. Journal code: NGP; 0125036. ISSN: 0026-8925. Pub. country: GERMANY, WEST: Germany, Federal Republic of. Language: English.

AB A cosmid gene bank of the virulent **Salmonella** typhimurium C5 was constructed in **Escherichia** coli K12. The bank was repackaged into bacteriophage heads and transduced into the semi-rough S. typhimurium strain AS68 which expresses the LamB lambda receptor protein. Approximately 6000 ampicillin-resistant transductants were pooled and used as host for the propagation of bacteriophage P22. The P22 lysate was able to transduce cosmid recombinants to smooth strains of S. typhimurium and individual transductants were selected which complemented various S. typhimurium auxotrophic mutations. A stable mutation was introduced into the **aroD** gene of S. typhimurium C5. The resulting **aroD** - mutant, named CU038, was highly attenuated compared with the wild-type parent strain and BALB/c mice immunised orally with CU038 were well protected against challenge with the virulent C5 parental strain. Using the cosmid bank repackaged into bacteriophage P22 heads it was possible to isolate cosmid recombinants that could complement the **aroD** mutation of CU038 either by in vitro selection using minimal medium or in vivo selection for restoration of virulence in BALB/c mice. Repackaged P22 cosmid banks could provide a simple system for selecting in vivo for **Salmonella** virulence determinants. A **Salmonella** typhi strain harbouring mutations in **aroA** and **aroD** was constructed for potential use as a live oral typhoid **vaccine** in humans.

DUPLICATE 31

L61 ANSWER 42 OF 42 MEDLINE
89067573 Document Number: 89067573. PubMed ID: 3058818. Construction and characterization of **vaccine** strains of **Salmonella** harboring mutations in two different aro genes. Dougan G; **Chatfield S**; Pickard D; Bester J; O'Callaghan D; Maskell D. (Department of Molecular Biology, Wellcome Research Laboratories, Beckenham, Kent, England.) JOURNAL OF INFECTIOUS DISEASES, (1988 Dec) 158 (6) 1329-35. Journal code: IH3; 0413675. ISSN: 0022-1899. Pub. country: United States. Language: English.

AB Derivatives of the mouse-virulent **Salmonella** typhimurium strain SL1344 were constructed harboring stable mutations in **aroC** alone or in **aroC** and **aroA** together. Fifty percent lethal doses after intravenous inoculation of the mutants into BALB/c mice were determined, and the mutants were as highly attenuated as were SL1344 **aroA** derivatives. All aro-dependent derivatives persisted in vivo at similar levels and for similar intervals in the livers and spleens of

US 096469250OP1

Creation date: 10-22-2003
Indexing Officer: DLO - DAT LO
Team: OIPEBackFileIndexing
Dossier: 09646925

Legal Date: 12-08-2002

No.	Doccode	Number of pages
1	SRNT	1

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